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Adenosine 5′-monophosphate increases levels of leukotrienes in breath condensate in asthma

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Summary Hyperresponsiveness (AHR) is a key physiological abnormality in asthma. In clinical and research studies AHR is measured bronchial challenge, with methacholine (MCh), but more recently with adenosine-5′-monophosphate (AMP).

In the search for markers of airway inflammation in asthmatic patients, we measured the concentrations of histamine and cysteinyl-leukotrienes (cys-LTs) before and after MCh and AMP challenges in the exhaled breath condensate of 13 patients with mild asthma (FEV_1 78.5%pred) and nine healthy non-smokers, using specific enzyme immunoassays.

With methacholine challenge we did not find any differences between asthmatics and normal subjects in the pre- and post-challenge concentrations of cys-LTs: 27.2 ± 1.4 vs. 29.2 ± 1.2 pg/ml and 26.3 ± 2.2 vs. 27.5 ± 4.2 pg/ml, respectively or histamine: 5.1 ± 0.4 vs. 5.1 ± 0.6 nM and 4.5 ± 0.4 vs. 4.4 ± 0.3 nM; $P>0.05$). In asthmatic patients cys-LT levels were significantly higher after AMP challenge (56.2 ± 9.7 vs. 31.7 ± 6.9 pg/ml; $P<0.05$); but there was no difference in healthy subjects (27.2 ± 4.6 vs. 30.3 ± 4.7 pg/ml). There was no difference in histamine concentrations in asthmatic (5.9 ± 1.8 vs. 4.5 ± 0.5 nM), or healthy subjects (5.5 ± 0.4 vs.5.7 ± 0.9 nM) after AMP challenge.

In conclusion, our results show that the cys-LTs are increased in exhaled breath condensate after AMP challenge, which may indicate that the AMP acts indirectly by releasing cys-LTs from primed mast cells. The detection of LTs and histamine in exhaled breath condensate may be useful in monitoring asthma.

Introduction

Asthma is an inflammatory disease of the airways associated with airway hyperresponsiveness (AHR) and variable airflow obstruction. The demonstration of AHR is widely advocated as a useful diagnostic test in the evaluation of possible asthmatics. AHR can be defined as an increased tendency of the airways to constrict excessively under the influence of various stimuli.[1] In clinical and research studies, AHR is measured by means of a bronchial challenge, usually with methacholine, histamine or adenosine-5′-monophosphate (AMP). Methacholine (MCh) and histamine act directly on airway smooth muscle, whereas AMP acts indirectly by causing primed mast cell degranulation and the release of proinflammatory mediators, such as histamine and leukotrienes.[2]

The inflammatory process in asthma involves multiple mediators, of which histamine was one of

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the first to be discovered; it is synthesised and released by mast cells in the airway wall and by circulating and infiltrating basophils. Recent evidence relating to the biochemistry, pathophysiology and antagonism of cysteinyl-leukotrienes (cys-LTs) suggests that they play a role in the pathophysiology of asthma.[3] Cys-LTs are produced predominantly by mast cells and eosinophils, large numbers of which are present in or recruited to the airways of asthmatic patients.

Airway inflammation can also be monitored by measuring non-invasive and sensitive markers, such as induced sputum [4] and exhaled nitric oxide (NO),[5] which may provide additional information for assessing asthma control. Exhaled breath condensate has also recently been used to assess inflammatory molecules derived from the respiratory tract, which it may contain. It is assumed that the airway surface liquid becomes aerosolised during turbulent airflow, and so the content of the condensate reflects the composition of airway surface liquid.[6]

The aim of this study was to investigate whether histamine and cys-LTs can be detected in the breath condensate of asthmatic patients and healthy controls before and after bronchoconstriction induced by MCh and AMP challenge. AMP is in asthma patients bronchoconstrictor and is assumed to cause bronchoconstriction indirectly by releasing bronchoconstrictor mediators, such as histamine or cys-LTs from mast cells.[2]

Methods

Study subjects

The study included 13 non-smoking asthma patients aged 24.4±2.6 years, and nine healthy controls without signs or symptoms of upper or lower respiratory disease. All patients had mild episodic asthma with forced expiratory volume in 1 s (FEV_1) of 83±1.4% of predicted value and a bronchodilator response to 200 μg of Salbutamol (FEV_1 increase >200 ml or 12% of baseline value). Their asthma was clinically stable and there had been no worsening in symptoms during the previous 8 weeks (Table 1).

The patients used only short-acting inhaled B-agonists to control their asthma during the 2 weeks before screening and no patient was taking inhaled corticosteroids. The study was approved by the Ethics Committee of the Royal Brompton Hospital and Harefield NHS Trust, and informed consent was obtained from all the patients.

Table 1 Physical characteristics and pulmonary function of subjects.

Parameters	Asthmatics	Healthy
Number	13	9
Age (yr)	24.4±2.6	33.2±9.6
Sex (F/M)	8/5	5/4
FEV_1 (L)	2.6±0.8	3.6±0.6
FEV_1 (% pred.)	83±1.4	97±12.3
FVC (L)	3.40±0.5	3.35±0.9
FVC (% pred.)	94.3±2.6	97±0.07
PC_{20} (MCh) mg/ml	4.5±1.7	>50
PC_{20} (AMP) mg/ml	12.2±2	>100

FEV_1 = forced expiratory volume in 1 s; FVC = forced vital capacity. PC_{20} = Mean values±SEM.

Study design

The patients were asked to visit the hospital on two occasions separated by an interval of at least 48 h in order to undergo MCh and AMP challenges in random order. The challenges were carried out after the baseline measurement of FEV_1 and the collection of exhaled condensate for 10 min, which was repeated immediately after the challenges.

Pulmonary function

Pulmonary function was measured using a dry spirometer (Vitalograph Ltd, Buckingham, UK); the mean of the three manoeuvres was expressed as an absolute value (in litres) and a percentage of the predicted value.

Inhalation challenge tests

Before the MCh and AMP challenges, the patients were instructed to withhold medications for the following time intervals: salbutamol for 6 h, oral antihistamines for 48 h, nasal sodium cromoglycate for 24 h, and intranasal anticholinergics and topical nasal decongestants for 48 h. They also had to avoid exercise or cold air exposure for at least 2 h and refrain from the ingestion of coffee, tea, cola and chocolate for at least 6 h before the challenge.

Methacholine chloride and AMP (Sigma Chemical Co., Poole, UK) were dissolved in 0.9% sodium chloride to produce a range of doubling concentrations (from 0.06 to 64 mg/ml for MCh and from 0.048 to 400 mg/ml for AMP) and then used immediately for bronchial challenge.

The doses were inhaled through a nebulizer attached to a breath-activated dosimeter (Mefar,

Brescia, Italy). A standard procedure was used for all of the provocation tests. Three values of FEV_1 were recorded at 1 min intervals and the highest value was taken as baseline. Subjects inhaled five 1-s breaths of normal saline as a control and held their breath for 6 s, FEV_1 was measured 2 min later. They then inhaled a series of doubling concentrations of MCh or AMP (starting with the lowest dose) at 3-min intervals and FEV_1 was measured 2 min after each dose. The challenges were stopped when FEV_1 fell by $>20\%$ from the post-saline value or the top dose was reached. A long dose–response curve was constructed and the concentration causing a 20% drop in FEV_1 from the post-saline value (PC_{20}) was calculated by means of linear interpolation and expressed in cumulative units.

Exhaled breath condensate

Exhaled breath condensate was collected immediately following the spirometry and after the last dose of bronchoconstrictor. The breath condensate samples were collected using a specially designed condensing chamber (Ecoscreen; Jaeger, Hoechberg, Germany); exhaled air enters and leaves the chamber through one-way inlet and outlet valves, thus keeping the chamber closed. The subjects wore nose clips and breathed tidally through a mouthpiece connected to the condenser for 10 min as previously described.[7] Approximately 1 ml of condensate was stored at $-70^{\circ}C$ in a 2-ml sterile plastic tube.

Histamine and cys-LT assays

Histamine and cys-LT concentrations in the breath condensate were measured using specific enzyme immunoassay (EIA) kits (Cayman Chemical, Ann Arbor, USA). The assays were validated directly, by means of gas chromatography/mass spectrometry in order to obtain a close correlation ($r=0.95$) between known amounts of histamine and cys-LTs and the concentrations measured by the EIA. The detection limit of the assay was 13 pg/ml for cys-LTs and 0.5 nM for histamine after a 2-h development period.

Statistical analysis

The data were expressed as mean values $\pm$ standard deviations, with significance being defined as a P value of <0.05. The intra-group differences were compared using Student's t-test for paired data and the differences between groups were compared using the Mann–Whitney U-test. The relationships between the clinical data and histamine and cys-LT concentrations in the breath condensates were investigated by means of Spearman's correlation.

Results

Methacholine challenge

There was no difference between asthmatic and healthy control subjects at baseline in histamine (5.1 ± 0.4 vs. 4.5 ± 0.4 nM; $P>0.05$) or cys-LTs concentrations (27.2 ± 1.4 vs. 26.3 ± 2.2 pg; $P>0.05$) in exhaled breath condensate.

Histamine was detectable in breath condensates of all asthmatic patients and control subjects, and its concentrations were unchanged after MCh challenge (5.1 ± 0.6 vs. 4.4 ± 0.3 nM; $P>0.05$, asthmatics and healthy, respectively, Fig. 1). Cys-LTs were detectable in 11/13 patients and in all healthy and similarly no difference was found after the challenge (29.2 ± 1.2 vs. 27.5 ± 4.2 pg/ml; $P>0.05$, Fig. 2).

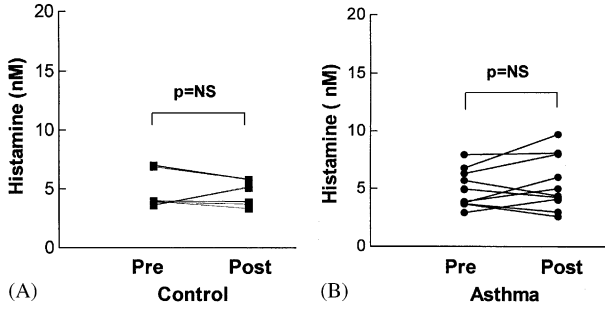

Figure 1 Exhaled breath concentrations of histamine in healthy control (Panel A) and patients with asthma (Panel B) after methacholine challenge.

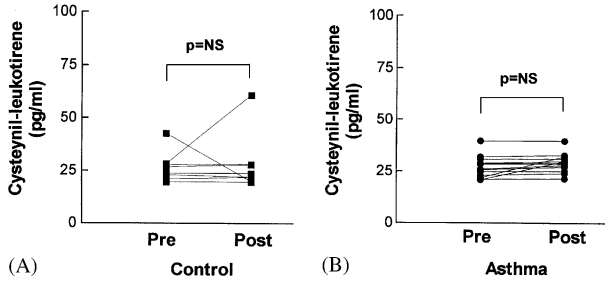

Figure 2 Exhaled breath concentrations of cys-LT in healthy control (Panel A) and patients with asthma (Panel B) after methacholine challenge.

AMP challenge

There was no difference between asthmatic and healthy control subjects at baseline in histamine (5.9 ± 1.4 vs. 5.5 ± 0.4 nM; $P > 0.05$) or cys-LTs concentrations (31.7 ± 6.9 vs. 27.2 ± 4.6 pg; $P > 0.05$) in exhaled breath condensate. Histamine was detectable in the breath condensate of all of the subjects and healthy, and its concentrations were unchanged after AMP challenge (4.5 ± 0.5 vs. 5.7 ± 0.9 nM; $P > 0.05$, Fig. 3).

Cys-LTs were detectable in 12/13 asthmatic patients and in all healthy subjects. We found a significant increase in cys-LTs concentrations after AMP challenge in asthmatics (from 31.7 ± 6.9 to 56.2 ± 9.7 pg/ml; $P < 0.02$, Fig. 4), but no change in healthy subjects from 27.2 ± 4.6 to 30.3 ± 4.7 pg/ml; $P =$ ns. There were no correlation between histamine and cys-LT levels and age, FEV_1 or PC_{20} in each groups of subjects.

Discussion

In asthmatic patients we found a significant increase in cys-LT concentrations in exhaled breath condensate after AMP challenge and a non-significant trend towards an increase after the MCh challenge, whereas histamine levels remained unaffected by either (Fig. 3). This suggests that the AMP may cause bronchoconstriction in asthma by releasing cys-LTs from airway mast cells.

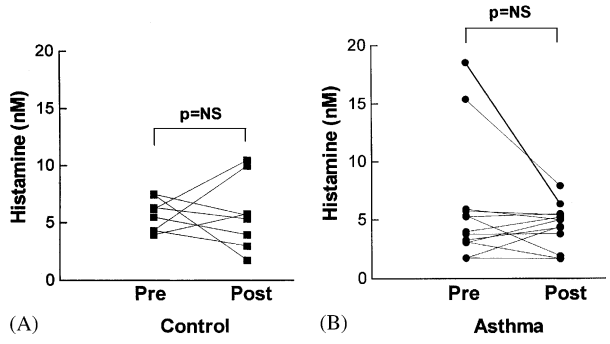

Figure 3 Exhaled breath concentrations of histamine in healthy control (Panel A) and patients with asthma (Panel B) after adenosine 5′-monophosphate challenge.

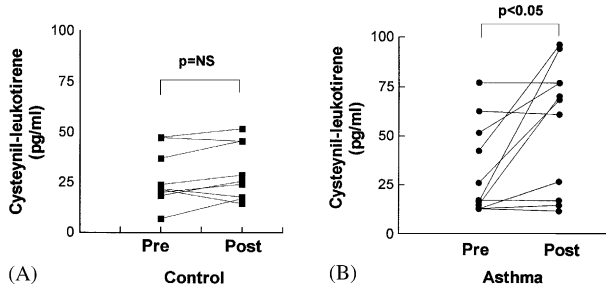

Figure 4 Exhaled breath concentrations of cys-LT in healthy control (Panel A) and patients with asthma (Panel B) after adenosine 5′-monophosphate challenge.

Cys-LTs are potent constrictors in human bronchi in vitro, being approximately 1000 times more potent than histamine and this effect is mediated via the activation of cys-LT receptors on airway smooth muscle cells. In addition, airways of asthmatics are may be usually more responsive to cys-LTs than those of healthy subjects, although the magnitude of the difference varies.[8] Cys-LTs are potent constrictors of both peripheral and central airway smooth muscle. Previous studies have found that the cys-LT release from blood leukocytes is greater in asthmatics than in controls,[9] and high levels of LTE_4 were found in the urine of patients suffering from spontaneous asthma attacks,[10] exercise-induced bronchospasm,[11] nocturnal asthma exacerbations,[12] and mild and moderate asthma in comparison to healthy subjects.

We did not find any change in cys-LT concentration in exhaled breath condensate after a MCh challenge, and believe that the PC_{20} AMP (which acts indirectly via the release of mediators from primed mast cells) more closely reflects cell activation in asthma patients than PC_{20} MCh, which acts on airway smooth muscle cells (Fig. 4). Van Den Berge et al.[13] found that the PC_{20} AMP is more closely associated with airway inflammation than PC_{20} MCh, and Polosa et al.[14] found an association between the number of eosinophilis in sputum and PC_{20} AMP (but not PC_{20} MCh) in 12 subjects with allergic rhinitis.

The fact that there was no change in histamine levels after MCh or AMP challenge was surprising. One probable explanation is that the changes in histamine concentrations is not manifest in detectable changes in exhaled breath condensate.

An other possible explanation is that the inhaled AMP releases cys-LTs from eosinophils rather than mast cells. However, there is no evidence for adenosine-induced activation of eosinophils in vitro,[15] so this is unlikely to account for the increase in cys-LTs, but not in histamine.

Our results show that the cys-LTs are increased in exhaled breath condensate after AMP challenge indicating that the AMP acts indirectly by releasing cys-LTs from primed mast cells. The detection of cys-LT in exhaled breath condensate may be useful as a means of detecting inflammatory cells in asthma.

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